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CONTRACTING ORGANIZATION: Children's Hospital
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13. ABSTRACT *(Maximum 200 words)*

The goal of this project is identify molecules that may be useful as predictive markers for human prostate cancer and to develop these molecules for use in clinically relevant tests that can predict outcome and disease course in prostate cancer patients. Our initial work has been focused on thymosin β15 (TB15), a molecule found in mid to high-grade prostate cancers. Our current results indicate that thymosin β15 is differentially expressed in invasive and in metastatic tumors but is less frequently expressed in non-invasive tumors. Expression of thymosin β15 at the time of diagnosis correlates with subsequent PSA failure, tumor recurrence, metastasis and mortality. We have also developed an ELISA for thymosin β15 and have used this test to detect the protein in the urine of prostate cancer patients. We believe that thymosin β15 along with other prognostic markers currently being developed could be useful in predicting outcomes and in choosing appropriate treatment strategies in prostate cancer patients.

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Bruce N Zeitlin 9/27/99

PI - Signature Date

Table of Contents

DAMD17-98-1-8591, Potential Prognostic Markers for Human Prostate Cancer

P.I. Bruce R. Zetter

5) INTRODUCTION:

Although the development of the PSA test has improved our ability to diagnose prostate cancer, this has not been accompanied by improvements in prostate cancer prognosis. The goal of this project is identify molecules that may be useful as predictive markers for human prostate cancer and to develop these molecules for use in clinically relevant tests that can predict outcome and disease course in prostate cancer patients. Our approach has been to identify molecules that are upregulated late in the course of prostate cancer progression and to then test whether the expression of these markers correlates with disease outcome. Our initial work has been focused on thymosin β15 (TB15), a molecule found in mid to high grade prostate cancers. We have been following two approaches to develop TB15 testing as a clinically useful approach. The first involves using immunohisto-chemistry to assay tissue sections from human prostates for the presence of TB15 and to then correlate the expression patterns with PSA failure, tumor recurrence, metastasis or mortality. The second approach has been to attempt to develop an ELISA test to detect TB15 in the urine or serum of prostate cancer patients in order to have a non-invasive assay. We have accomplished both of these goals and our early results do suggest that TB15 may have promise as a marker that can predict later tumor aggressiveness and metastatic potential. We believe that such a test could eventually be used to distinguish those patients at low risk of recurrence from those who are at higher risk. This information could be extremely useful in helping patients and their physicians to choose between the wide range of treatment options currently available.

6) BODY:

In previous studies, by comparing gene expression among the Dunning R-3327 rat prostatic adenocarcinoma variants (1), we cloned a novel β-thymosin gene, thymosin β15, which was expressed in highly metastatic variants, but not in poorly metastatic variant (2). The thymosin β family comprises very small, highly conserved and acidic proteins existing in many different animal species (3). The most abundant member of the family, thymosin β4, which was originally isolated from calf thymus and postulated to play a role in thymic immune development, is present in all mammalian species, and along with the related family member thymosin β10, is widely distributed in a variety of cell types. The main function of thymosins β4 and β10 is to bind monomeric actin and to retard actin polymerization (4)(5). Thymosin β15 also binds monomeric actin and appears to regulate cell motility as transfection of antisense thymosin β15 into rat prostatic carcinoma cells can significantly reduce stimulated cell migration (2).

When we investigated the pattern of expression of TB15 in human prostate cancer we found that low-grade prostate cancers (Gleason 3-5) generally did not express TB15 whereas high-grade tumors (Gleason 8-10) most often had high TB15 levels. What was most interesting, however, was that there was no discernable pattern of TB15 expression in mid-grade tumors. Some were positive for TB15 and some were negative. This raised the prospect that TB15 might represent a predictive marker in human prostate cancer, i.e., that tumors that expressed TB15 might be more aggressive than tumors in which TB15 was not expressed. In Task 1 of this proposal, we further evaluated thymosin β15's use as a potential biomarker that can function as an indicator of metastatic progression and disease outcome in human prostate carcinoma patients by a small-scale retrospective study.

Task 1. Continued Development of Thymosin β15 as a Prognostic Marker in Human Prostate Cancer.

Goals:

a. Continue to accumulate follow-up data on the original cohort of patients analyzed for TB15 expression in 1995/6 (months 1-30).

b. Collect and stain tissue specimens for a large (>200 patient) retrospective study correlating TB15 expression with tumor metastasis and patient outcome (Months 6-24).

Results:

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin β15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/thymosin β fusion proteins with the affinity-purified anti C-terminal antibody. The purified antibody reacted strongly with the GST-thymosin β15 fusion protein, but did not cross react with GST-thymosin β4, nor with GST alone.

We used the affinity purified polyclonal thymosin β15 antibody for immuno-histochemical study of 150 human prostate carcinoma cases. Positive immunostaining was observed in the cytoplasm of carcinoma cells in neoplastic prostates but not in normal prostates and not in the stromal cells (Figure 2A). Among the specimens investigated, poorly differentiated adenocarcinomas with Gleason scores 8-10 displayed the most extensive and intense thymosin β15 immunoreaction, followed by moderately differentiated prostate carcinomas with Gleason scores of 6-7 in which some but not all carcinomas were TB15 positive. In some cases, specimens of PIN showed thymosin β15 immunostaining, but usually to a lesser extent than the malignant lesions. In poorly

differentiated and invasive prostate carcinoma, single cells invading the tissue stroma displayed intense staining. Well-differentiated carcinomas with Gleason scores generally showed no thymosin β15 staining or very low levels of staining.

Thymosin β15 staining levels for all prostatic carcinoma specimens are summarized in Figure 1. Specimens were scored as negative if less than 10% of the tumor tissue in each section showed staining of tumor cells, scored as positive (+) if staining was between 10% and 50%, and strong positive (++) if greater than 50% of the tumor tissue was stained. The results show a general correlation between thymosin β15 staining and the Gleason scores. In most cases, high-grade tumors (Gleason scores 8-10) have a higher percentage of positive staining than low-grade (2-5) tumors. Interestingly, moderately differentiated prostate carcinomas with Gleason scores (6-8) could be divided into three groups according to the levels of thymosin β15 expression. 17 (32%) out of 54 cases showed no expression of thymosin β15, about 50% of cells in 24 (44%) cases expressed thymosin β15, showing partial positivity, while 13 (24%) cases showed high levels of thymosin β15 expression (more than 75% cells were positive). These results suggest that thymosin β15, a potential marker of aggressive prostatic carcinoma, assorts independently in mid-grade prostatic carcinomas.

To further investigate whether TB15 expression correlated with invasion and/or metastasis in human prostate cancer, we conducted a study in collaboration with Dr. John Petros of Emory University Medical School. Tissue samples taken from radical prostatectomy were analyzed for TB15 staining and the staining level (negative -, positive +, strongly positive ++) was recorded and then compared with the invasive or metastatic state of the tumor at the time of prostatectomy. Of the patients with non-invasive disease, 8 were negative, 2 positive and 1 strongly positive for TB15 expression

as determined by immunohistochemistry. Patients with invasive but non-metastatic tumors were more generally positive with only 1 of 7 invasive tumors being negative for TB15, 4 positive and 2 strongly positive (Figure 2). Finally, all of the 15 patients with metastatic disease diagnosed at the time of surgery were either positive (7 patients) or strongly positive (8 patients).

The follow-up for three years or more on 26 of patients is summarized in Figure 2. Most striking is the data for the nine patients who have died of metastatic prostate cancer (DOD) in the past 3-5 years. Of these, none were negative, one showed positive staining and 8 displayed strongly positive for thymosin β15 at the time of diagnosis. Of the 15 patients still alive with no evidence of disease (NED) 3-5 years following diagnosis, 8 were negative for thymosin β15, three were positive and four were strongly positive for thymosin β15 staining. Thus, of the patients who have follow-up data, all of those patients who were negative for thymosin β15 staining at the time of diagnosis are still alive with no evidence of disease. Because the population is still only shortly removed from their initial diagnosis, it will be most interesting to monitor whether those patients with no current recurrence but with extensive thymosin β15 staining are indeed at greater risk to develop recurrent disease than those with low thymosin β15 staining.

Task 2. Development of an assay to detect thymosin β15 in human fluids such as urine or serum.

Goals:

a. Develop additional antibodies for TB15 (Month 1-6)

b. Develop a sensitive ELISA or sandwich ELISA assay for TB15

c. Determine whether TB15 can be detected in human fluids (Month 6-12)

d. If TB15 is detected in human fluids, attempt to correlate TB15 expression in patient serum or urine with tumor metastasis and patient outcome (Months 12-30).

It is clear that the utility of the TB15 test would be increased if a non-invasive assay could be developed that could detect TB15 levels in the serum or urine of patients with prostate cancer. The β thymosins are, however, intracellular actin-binding proteins and they lack a signal peptide for export making it less likely that they would be secreted into human body fluids. Interestingly, though, significant levels of thymosin β4 are found in the serum of normal individuals (6) suggesting that members of the β-thymosin family may be released into the circulation. We have therefore attempted to develop an ELISA for thymosin β15 and to use it to detect TB15 in the urine of prostate cancer patients.

Results:

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin β15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into both rabbits and chickens. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/thymosin β fusion proteins with the affinity-purified anti C-terminal antibody. The purified antibody strongly reacted with GST-thymosin β15 fusion protein, but did not cross react with GST-thymosin β4, nor with GST alone.

Using the chicken anti thymosin β15 antibodies, we have developed an ELISA to TB15 that can detect TB15 at concentrations of 10-20 ng/ml. The ELISA protocol is as follows:

TB15 (20 ng/well in 200 μl) was adsorbed to Costar high-binding 96 well plates for 2 h at 37C. The wells were then washed with buffer containing 3 mg/ml BSA and then

replaced with 200 µl of a solution containing a 1:2000 dilution of chicken anti-TB15 antibody along with samples containing urine from prostate cancer patients or from normal controls that had been pre-incubated overnight at 4C. The plates were incubated for 1 h at 37C, then washed 3X, incubated for an additional 1 h with rabbit anti-chicken IgG and finally developed with the Vectastain ABC reagent. A standard curve from a typical assay is shown in Figure 3. Increasing concentration of TB15 added to the antibody solution results in a progressive decrease in signal with concentrations from 20 ng/ml – 1250 ng/ml.

Our studies have now revealed that TB15 can be detected in the urine of some prostate cancer patients. Figure 4 shows some of the raw data for a group of 11 prostate cancer patients. We have now conducted assays on 120 prostate cancer patients. The only criterion for inclusion in the study was that the patient had been diagnosed as having prostate cancer and was under active care by a urologist. Time following diagnosis ranged from days to decades. Positive TB15 values are ascribed to any patients whose urine TB15 level equals or exceeds 40 ng/ml.

As shown in Figure 5, normal controls are generally negative with 31 patients negative and only 2 positive. Patients previously diagnosed with prostate cancer but disease-free at the time of urine collection were variable in TB15 production with 39 patients positive and 27 patients negative. Since many of these patients were only recently diagnosed, some of these individuals may go on to develop recurrent disease. Of 20 patients who had failed treatment as judged by increasing PSA levels at the time of urine collection, 18 had positive levels of TB15 in their urine whereas only 2 were negative. Finally of three patients who were bone scan positive, 2 were TB15 positive and one was TB15 negative.

We are pleased and surprised that we can detect TB15 in the urine of prostate cancer patients and we plan to perform similar studies on a larger patient sample. Certainly the trend appears to be that patients with tumor failure have an increased level of detectable TB15 in their urine. We are now trying to duplicate this work using patient serum as well as urine samples. Most importantly, we wish to follow this current cohort of patients over time to determine whether newly diagnosed patients who have the highest circulating TB15 levels will be more likely to develop recurrent disease at some later time.

Task 3.

Goals:

Develop new antibodies for the collagen-like protein and obtain antibodies for lipocalin-2 and other potential prognostic markers from investigators (Months 6-12).

Correlate expression of these additional prognostic markers in human prostate cancer tissue specimens with tumor metastasis and patient outcome (Months 12-24).

Develop a sensitive ELISA assay for detection of the collagen-like protein in patient serum and urine (Months 18-24).

If possible, correlate production of the collagen-like protein in patient serum and urine with tumor metastasis and patient outcome (Months 24-30).

Results:

Work on most of Task 3 is just commencing as most of these studies are scheduled to commence during the next year. We have, however, made some progress with our studies on a novel collagen-like gene that is upregulated in certain metastatic prostate cancer cell lines. This molecule has substantial collagen-like repeats along with

novel intervening sequences. We have made peptide antibodies to this molecule in chickens using methods similar to those described above for thymosin β15. Using these antibodies in immunohistochemistry on human prostate cancer sections, we find that the protein encoded by this new collagen-like gene (CLG) is also upregulated in aggressive prostate cancers.

As shown in Figure 6, the CLG protein is detected in predominantly in higher-grade prostate tumors (Gleason 6 and higher) with the most extensive staining in tumors with Gleason scores of 8 or higher). Further work is being conducted to extend this study and to correlate these findings to patient outcome. We will also attempt to develop an ELISA for CLG that can be used to detect the protein in patient fluids.

7) KEY RESEACH ACCOMPLISHMENTS

- ✧ Identified novel potential prognostic markers for human prostate cancer.
- ✧ Developed specific antibodies for potential prognostic markers.
- ✧ Showed that thymosin β15 expression correlates with invasiveness and metastasis in human prostate cancer.
- ✧ Showed that thymosin β15 expression correlates with patient outcome in human prostate cancer.
- ✧ Developed a competitive immunoassay for detection of thymosin β15.
- ✧ Demonstrated that thymosin β15 was present in the urine of patients with recurrent prostate cancer.
- ✧ Identified a novel collagen-like gene as a potential prognostic marker in human prostate cancer.

8) REPORTABLE OUTCOMES

Bao L, Loda M, Zetter BR. Thymosin β15 as a prognostic marker for human prostate cancer. Abstracts of the Workshop on Thymosin Peptides, 1999

Bao L, Zetter BR. A novel molecule with multiple domains of Gly-Xaa-Yaa repeats is upregulated in metastatic prostate carcinoma. Abstracts of the VII International Congress of the Metastasis research society, 1999.

9) CONCLUSIONS

Over the past year, we have made significant progress in developing a new prognostic assay for human prostate cancer. Thymosin β15 (TB15) was originally detected in a differential display screen designed to find genes that were upregulated in the later stages of tumor progression. Our studies now show that TB15 expression is extensively upregulated in prostate tumors that are invasive and/or metastatic. By determining the extent of TB15 staining in tumor sections taken at the time of original diagnosis, one can obtain an indication of the likelihood that the tumor will progress to the metastatic state. We have also been able to develop an ELISA for TB15 and have shown that we can detect this protein in the urine of patients with prostate cancer; in particular patients with recurrent prostate cancer. We are undertaking a prospective study to determine whether urinary TB15 levels at the time of diagnosis can also be predictive of future outcome. Finally, we have begun to develop a collagen-like protein that is upregulated in metastatic prostate cancer as a second prognostic marker. We believe that a panel of such agents will eventually provide an accurate prediction for the likelihood of failure, recurrence or metastasis in newly diagnosed patients.

APPENDIX

Figure legends:

Figure 1. Correlation of TB15 staining with invasion and metastasis. Tissue sections from radical prostatectomy specimens were obtained from Dr. John Petros, Emory University. TB15 expression was quantified according to the extent of the tumor tissue that showed positive staining with <10% staining considered negative, 10-50% staining positive (+) and >50% staining as strongly positive (++). Staining levels were then correlated with the status of the tumor at the time of surgery.

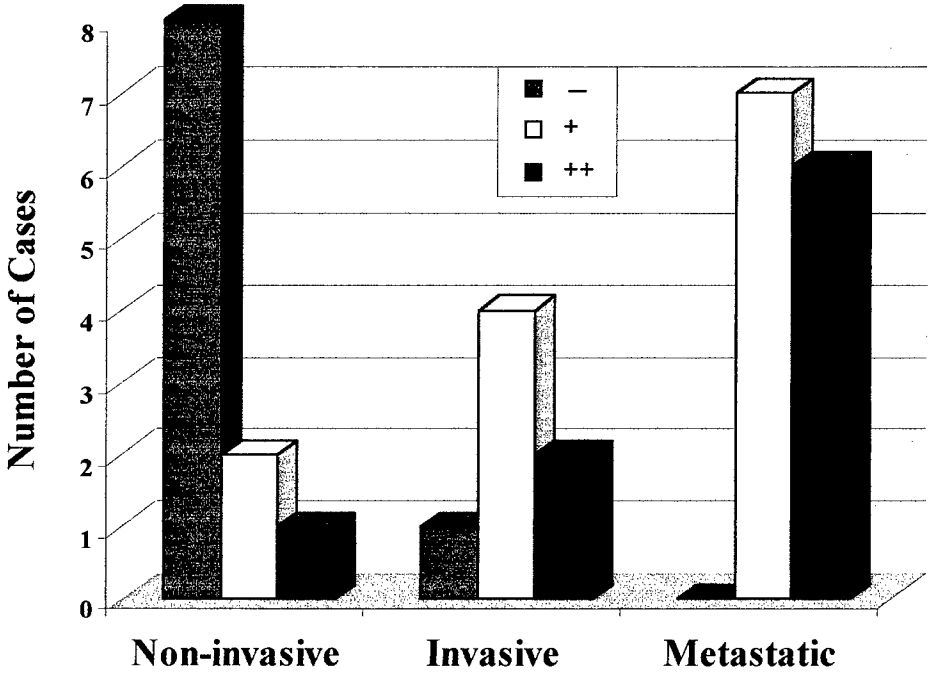
Figure 2. Correlation TB15 staining with tumor recurrence and patient survival status. TB15 staining in sections from radical prostatectomy are correlated with patient disease status 3-5 years later. In this sample, positive staining in seen in all patients with recurrent tumor as well as those who have died from the consequences of their tumor.

Figure 3. Competitive ELISA for TB15. Dose response curve for a competitive ELISA for thymosin β15. The curve is linear from 20 ng to >600 ng.

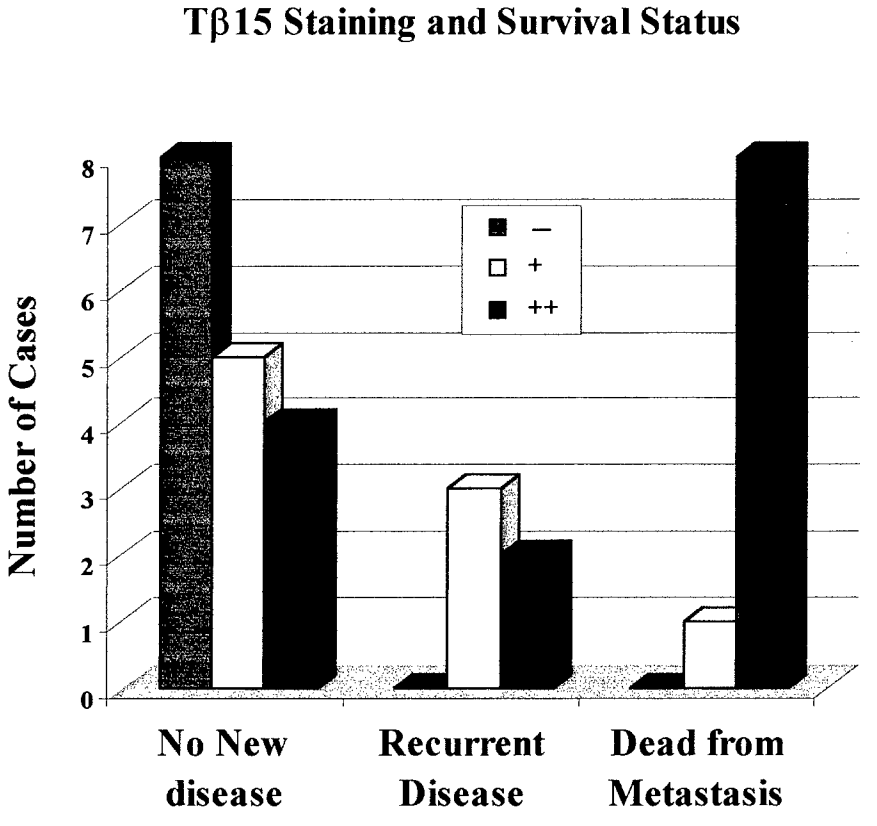
Figure 4. Detection of thymosin β15 in patient urine. Raw data showing a single dose response curve along with patient urine samples tested on the same day. Positive levels of TB15 are found in samples from patients 92, and 94-101. Patients 91, 93 and the normal control are considered negative.

Figure 5. Correlation of Urinary TB15 levels with patient status at the time of analysis. The sample represents a random population of prostate cancer patients, some of whom are newly diagnosed and others who are several years post-diagnosis. The significant findings are the lack of urinary TB15 levels in normal controls and the presence of TB15 in the urine of nearly all the patients with PSA failure.

Figure 6. Correlation of collagen-like gene (CLG) expression with Gleason score in human prostate cancer. CLG expression was measured by immunohistochemistry in tissue sections of radical prostatectomies. The results show a general correlation between CLG expression and increasing Gleason score.

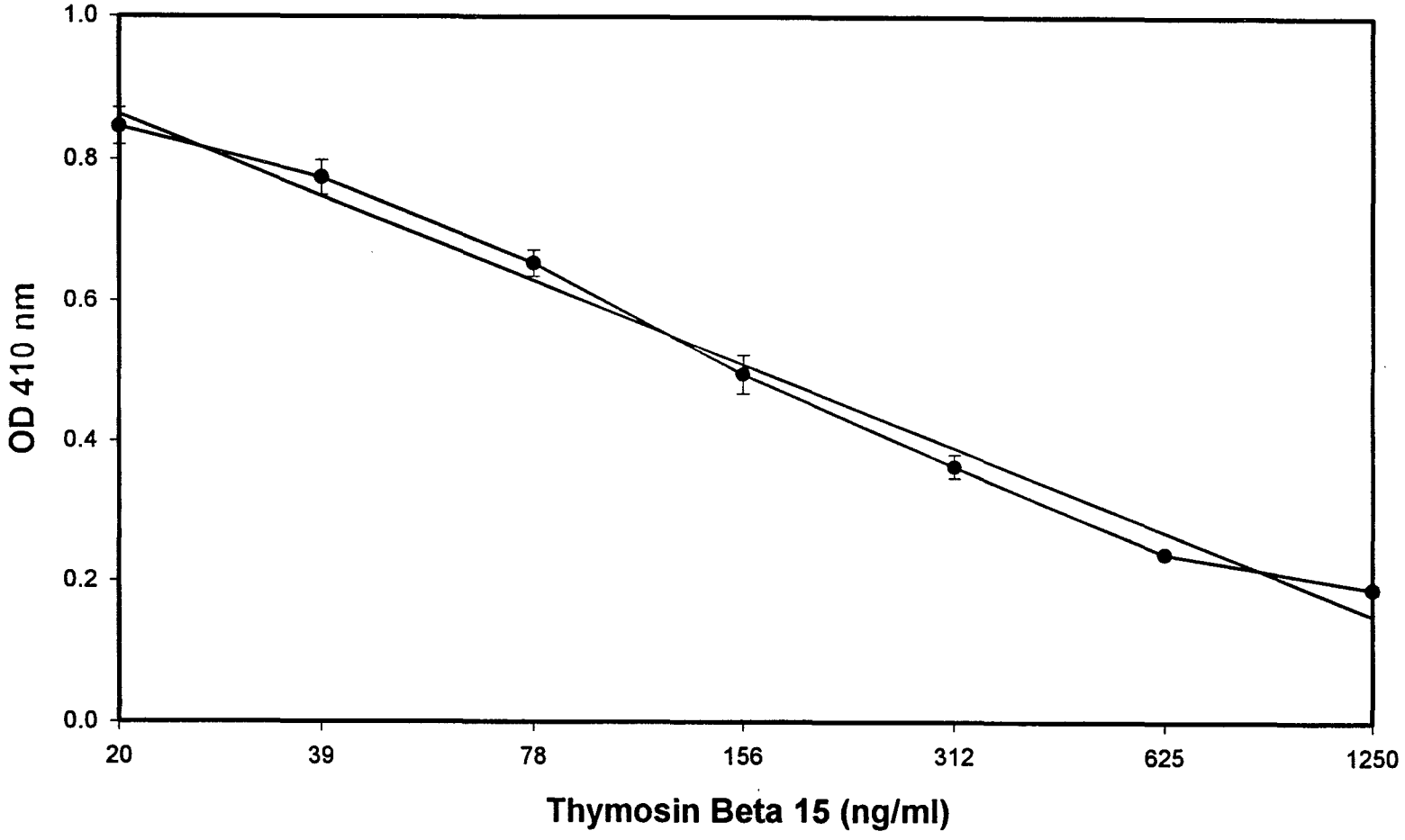


Zetter-Figure 1



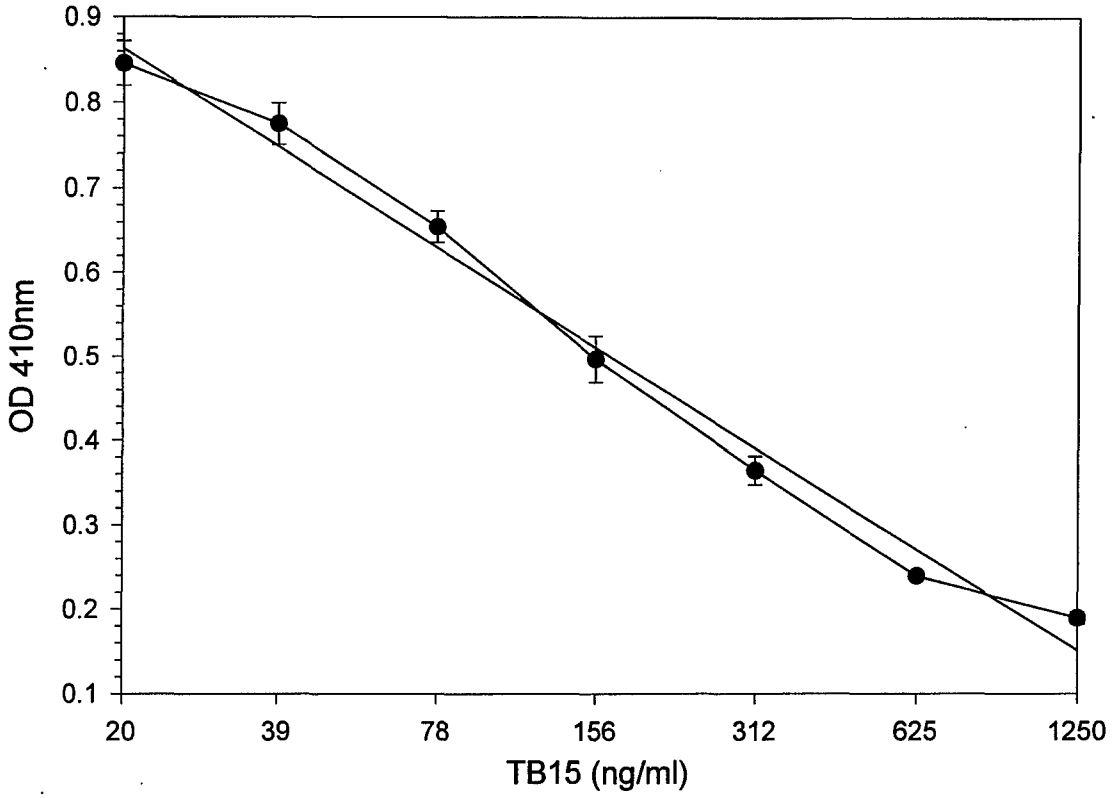
Zetter-Figure 2

Dilution Series of Thymosin Beta 15

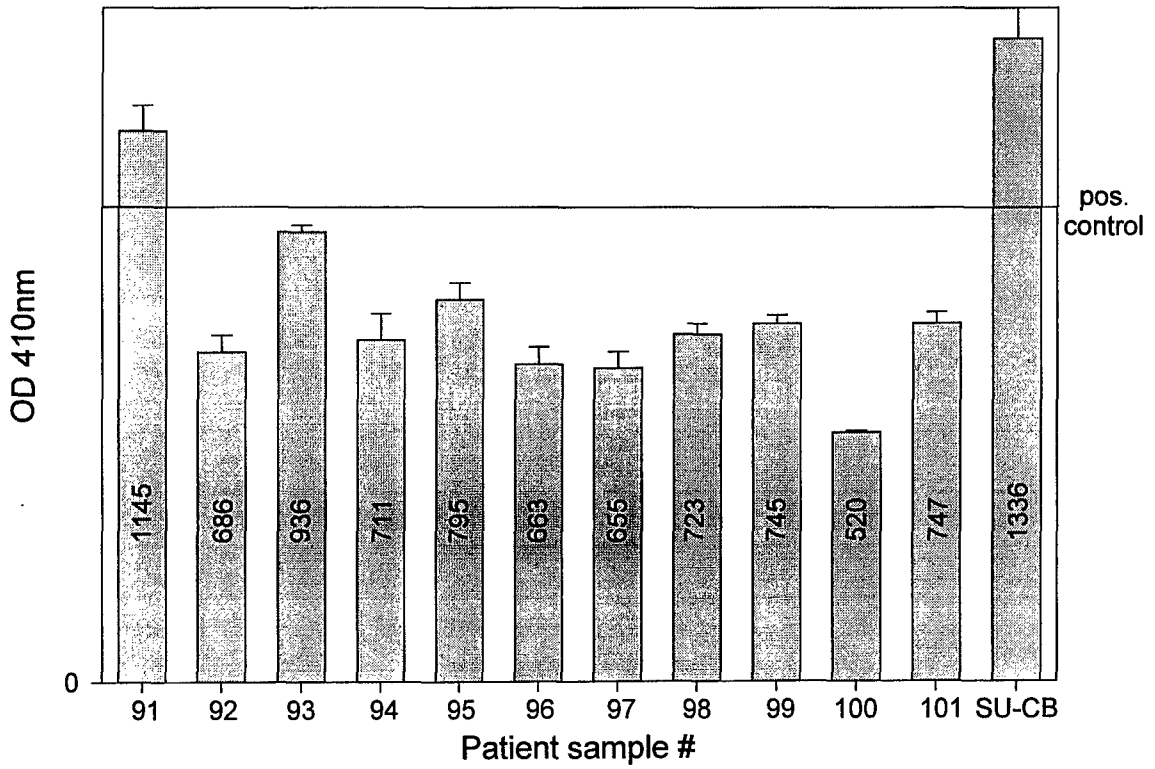


Zetter-Figure 3

ELISA # 20
Dilution series of TB15 in PBS

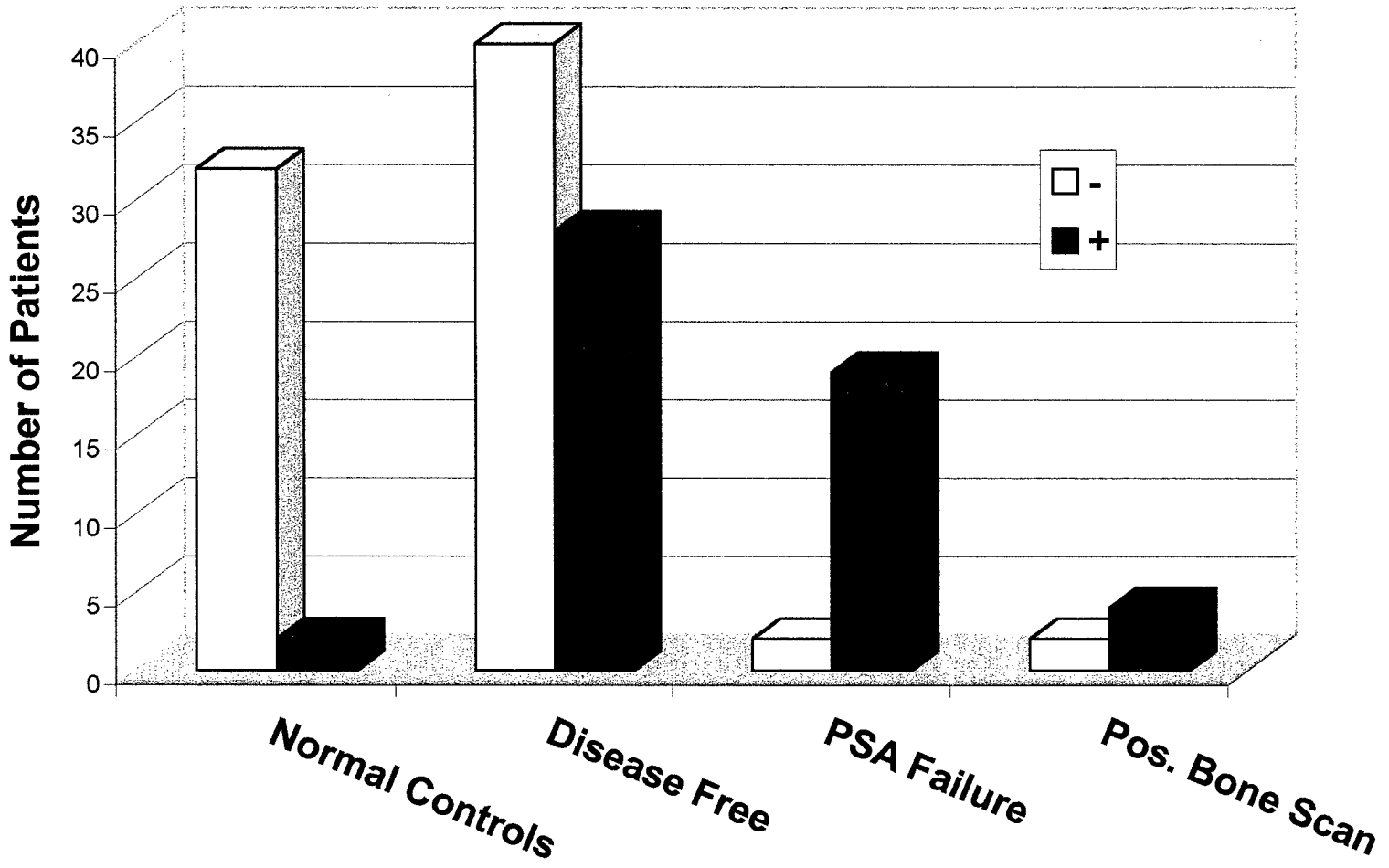


ELISA # 20
Standard urine,
Patient samples # 91 - # 101

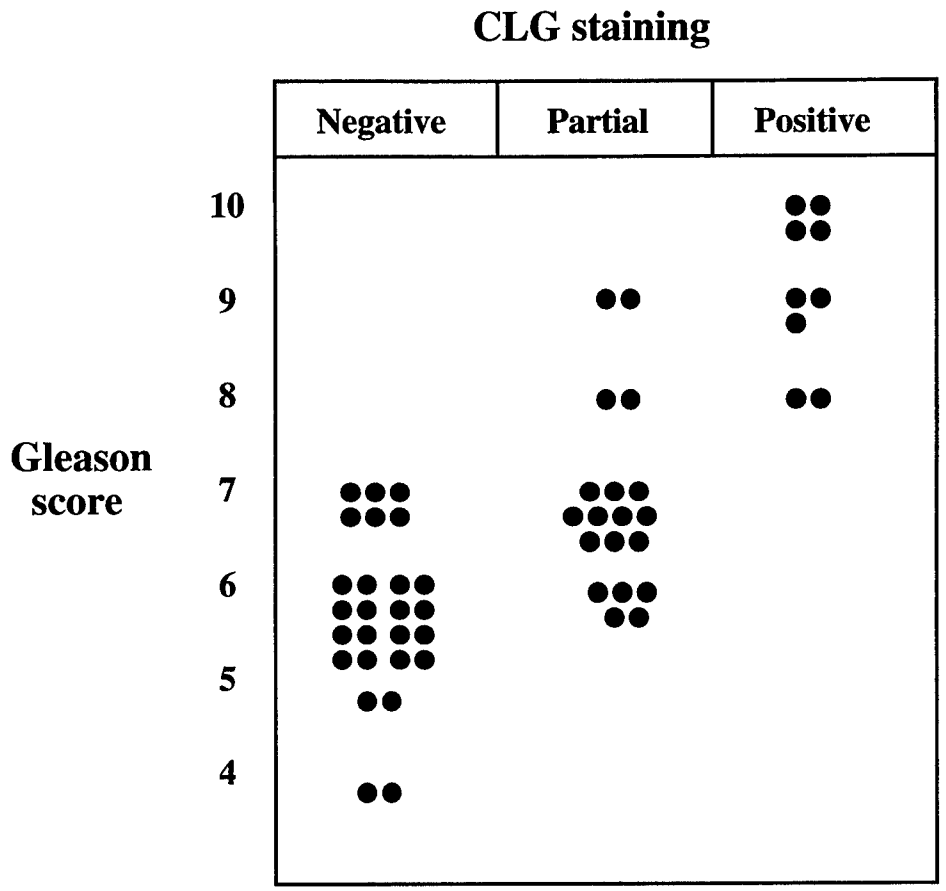


Zetter-Figure 4

ELISA of Thymosin Beta 15 in Urine of Prostate Cancer Patients



Zetter-Figure 5



Zetter-Figure 6

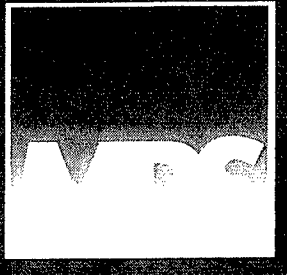
Metastasis Research Society

Programme and Abstracts

VII International Congress of the Metastasis Research Society

7–10 October 1998

San Diego, California, USA



associated with metastasis of carcinomas. We recently reported that inactivation of RB family proteins by SV40 T large antigen (LT) in MDCK epithelial cells results in a mesenchymal conversion associated with invasiveness and a down-regulation of c-myc. Re-expression of RB or c-myc in such cells allows the re-expression of epithelial markers including E-cadherin. Now we show that both RB and c-myc specifically activate transcription of the E-cadherin promoter in epithelial cells but not in NIH 3T3 mesenchymal cells. This transcriptional factor activity is mediated in both cases by the developmentally regulated transcription factor AP-2. *In vitro* AP-2 and RB interaction involves the N-terminal domain of AP-2 and the oncoprotein binding-domain and C-terminal domain of RB. *In vivo* physical interaction between RB and AP-2 was demonstrated in MDCK and HaCat cells. In MDCK(LT) cells LT, RB and AP-2 were all coimmunoprecipitated by each of the corresponding antibodies and a mutation of the RB binding domain of the oncoprotein inhibits both its binding to RB and AP-2. Taken together, our results suggest that there is a tripartite complex between LT, RB and AP-2 and that the physical and functional interaction between LT and AP-2 are mediated by RB. Moreover they define RB and c-myc as coactivators of AP-2 in epithelial cells and shed new light on the significance of the LT-RB complex, linking it to the dedifferentiation processes occurring during tumor progression. These data raise the possibility that one of the primary biological effects of RB and c-myc may be to positively regulate cellular genes involved in epithelial differentiation and that inactivation of this function may play a major role in tumor progression.

PA4.18

Modulation of CD44 expression in keratinocyte cell cultures by cytokine treatment

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Invasion and metastasis are great obstacles to successful tumor treatment. The multistep process of metastasis is influenced by different interactions between cells and matrix and various proteins respectively. Splice variants of the transmembrane glycoprotein CD44 seem to be correlated with advanced stages of tumor growth and metastatic potential. According to the results of Kaufmann *et al.* (1995) and Dall *et al.* (1994), the exons v6, v7 and v8 respectively are favoured for a diagnostic marker in cervical and mammary cancer tissue. We found high expression of all CD44 variants in cervical cancer cells but no over-expression of a single variant. CD44 as a differentiation-dependent adhesion molecule is regulated by various growth factors. For a better understanding of functional backgrounds of CD44 expression we investigated keratinocyte cell cultures after cytokine treatment. We sought to determine whether a specific CD44 variant is influenced by epidermal growth factor (EGF), keratinocyte growth factor (KGF), interferon c (1Fc) and tumor necrosis factor α (TNFα). The present experiments were analyzed by flowcytometry, immunocytochemistry and Southern blot hybridization with DIG-labeled exon-specific probes. Normal human epidermal keratinocytes expressed CD44 variant exons v3–v10 and CD44 standard, respectively, as analyzed by PCR-amplification and Southern blot hybridization. Immunocytochemical analysis demonstrate two patterns of CD44 isoforms on keratinocytes. CD44v3, v5 and v6 were expressed at a high level at cell-cell-contacts. On the other hand, CD44v4, v7/8 and v10 were expressed at a low level forming clusters spread over the cell membrane but sparing cell–cell contacts. Despite cytokine modulation the expression kept almost unchanged. The present study confirms a time-dependent regulation of CD44 variant expression by cytokine treatment. We postulate that various differentiation-dependent molecules might be involved in a signalling pathway leading to an alteration in CD44 expression after growth factor binding. This study was supported by Deutsche Krebshilfe (70-2036-Wo 1).

PA4.19

1α25 $(OH)_2D_3$ suppresses the expression of VLA-4 on the surface of HL 60 and A375 cells

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Department of Geriatrics, Endocrinology and Metabolism, Shinshu University School of Medicine, Matsumoto, Japan

The integrins, a large family of homologous transmembrane linker proteins, widely express on animal cells for binding most extracellular matrixproteins. VLA-4, a heterodimer complex of integrin α4 and β1, binds to vascular cell adhesion molecule-1 (VCAM-1) or extracellular fibronectin. It is reported that the anti-α4 integrin antibody suppresses the frequency of metastasis in the melanoma cells. 1α25$(OH)_2D_3$, the activated form of vitamin D3, induces differentiation of HL60 cells, including the increment of integrin β2 expression. Here we demonstrate that 1α25$(OH)_2D_3$ decreases the expression of either integrin α4 or β1 on the surface of human leukemic HL60 and melanoma A365 cells. The flow cytometric analysis shows that the expressions are suppressed in a dose dependent manner, and the maximal suppression was obtained 5 days after incubation with vitamin D3. Whereas, the expression of α4 integrin mRNA was not suppressed within 5 days after incubation with 10^{-6} M vitamin D3 in HL60 cells. These findings indicate that the activated vitamin D3 suppresses the expression of α4 integrin at post-translational level, resulting in the diminished expression of VLA-4. It is reasonable to postulate that vitamin D3 inhibits cell attachment in susceptible tissues and that this accounts for its anti-metastatic activity.

PA4.20

A novel molecule with multiple domains of Gly-Xaa-Yaa repeats is upregulated in metastatic prostate carcinoma

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Identification of quantitative changes in gene expression that occur in high-metastatic versus non- or low-metastatic tumor cells is important for understanding the molecular basis of cancer metastasis. Using differential mRNA display, we have isolated a cDNA fragment from Dunning R-3327 rat prostatic adenocarcinoma cell variants. The isolated cDNA fragment represents mRNA that was expressed in high-metastatic variant AT6.1, but not in low-metastatic variant AT2.1. The expression pattern shown by differential mRNA display was confirmed by Northern analysis. The cDNA fragment was used as a probe to screen an AT6.1 λgt10 cDNA library that resulted in isolating a positive clone with 2.5 kb insert. Nucleotide sequence analysis shows the cDNA encode a N-terminus truncated collagenous polypeptide that was not identical to any known collagens. The new collagen contains 3 triple-helical domains separated and flanked by non-triple-helical regions. Immunohistochemical studies of human prostate carcinoma samples using a polyclonal antibody prepared against the GST fusion protein of C-terminus of the new collagen reveal that the positive staining correlates with a well characterized indicator of tumor progression, the Gleason grade of the tumor. These results suggest that this new molecule may represent a potentially new biochemical marker for advanced human prostate cancer.

PA4.21

Adhesive interactions of human colon carcinoma cells expressing sialyl LeX carbohydrate chains with mouse liver sections

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ABSTRACTS BOOK

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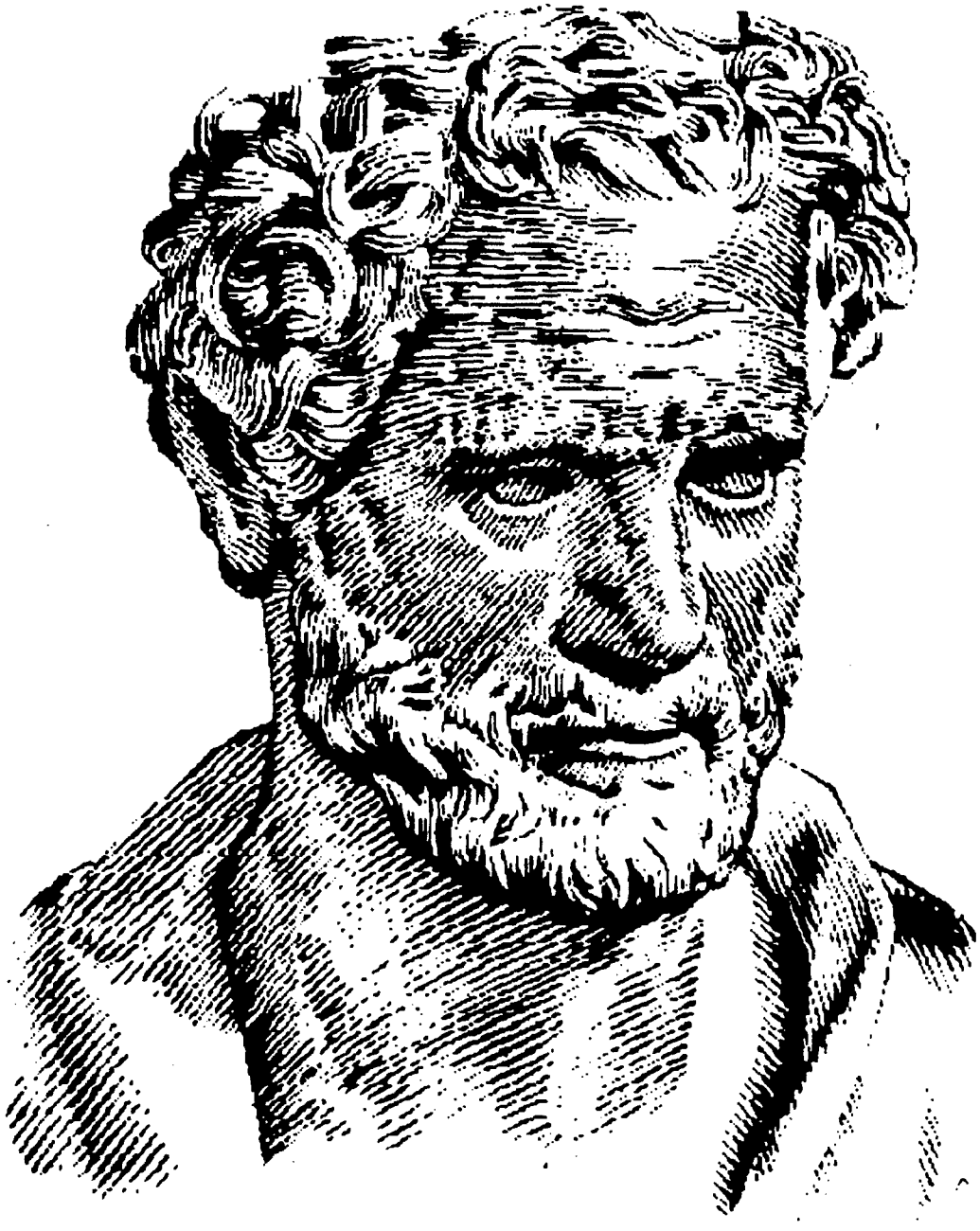

Workshop on Thymosin Peptides:

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Greece, July 1-2, 1999

THYMOSIN β15 AS A PROGNOSTIC MARKER FOR HUMAN PROSTATE CANCER

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Prostate cancer is the commonest cancer in men and the second leading cause of cancer death in American men. The widespread use of prostate specific antigen (PSA) test has greatly improved the earlier detection of human prostate cancers. However, this early detection does not help much to predict which tumors will progress to the metastatic diseases. To search for molecules that could be used as prognostic markers for prostate cancer, we compared gene expression among Dunning rat prostatic carcinoma cell lines with varying metastatic potential and cloned a gene called thymosin beta 15 (TB15), a new member of the thymosin beta family, from a metastatic subclone. TB15 was not detected in most normal adult rat and human tissues, including prostate. *In situ* hybridization and immunohistochemical staining of human prostate specimens showed that both TB15 mRNA and protein levels were elevated in invasive and metastatic tumors and correlated positively with the Gleason grade, a common histological grading system of prostate cancer. Up to 5 years follow-up data from the patients we could obtain showed that all patients who died of metastatic prostate cancer had positive staining for TB15 at the time of diagnosis. In contrast, patients who were negative for TB15 are still alive with no evidence of disease. These data suggest that TB15 could serve as a molecular marker that is able to distinguish prostate cancers destined to progress to lethal metastatic disease from those with little likelihood of causing morbidity.

DEPARTMENT OF THE ARMY
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